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(54) Title: REGULATORS OF INTRACELLULAR PHOSPHORYLATION

(57) Abstract

The invention provides human regulators of intracellular phosphorylation (HRIP) and polynucleotides which identify and encode HRIP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HRIP.

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REGULATORS OF INTRACELLULAR PHOSPHORYLATION

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of regulators of intracellular ph sphorylation and to the use of these sequences in the diagnosis, treatment, and prevention of neurological, cell proliferative, and autoimmune/inflammatory disorders.

BACKGROUND OF THE INVENTION

Reversible protein phosphorylation is the main strategy for controlling the activities of eukaryotic cells. Kinases and phosphatases regulate reversible phosphorylation reactions, and are thus critical components of intracellular signal transduction pathways. Protein kinases transfer the high energy phosphate from adenosine triphosphate (ATP) to specific protein targets in response to extracellular signals (such as hormones, neurotransmitters, and growth and differentiation factors), cell cycle checkpoints (for example, signals associated with mitotic events), and environmental or nutritional stresses. Protein phosphatases mediate kinase effects by removing phosphate groups from proteins.

It is estimated that more than 1000 of the 10,000 proteins active in a typical mammalian cell are phosphorylated. In general, protein activity is stimulated by phosphorylation, and this is analogous to turning on a molecular switch. When the switch is turned on, the appropriate protein kinase activates a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, or transcription factor. Protein activity is repressed by dephosphorylation when down-regulation of a signaling pathway is required. The coordinate activities of kinases and phosphatases regulate key processes such as cell proliferation, cell differentiation, cell-cell communication, and cell cycle progression. Uncontrolled signaling has been implicated in a variety of disease conditions including inflammation, cancer, arteriosclerosis, and psoriasis.

Protein kinases phosphorylate protein acceptor molecules on hydroxylated amino acids. These kinases comprise the largest known protein group, a superfamily of enzymes with widely varied functions and specificities. Protein kinases are usually named after substrates, regulatory molecules, or some aspect of a mutant phenotype. With regard to substrates, protein kinases may be roughly divided into two groups: those that phosphorylate tyrosine residues (protein tyrosine kinases (PTKs)), and those that phosphorylate serine or threonine residues (serine/threonine kinases (STKs)). A few protein kinases have dual specificity and phosphorylate serine, threonine, and tyrosine residues. Some STKs and PTKs possess structural characteristics of both families (Hardie, G. and S. Hanks (1995) The Protein Kinase Facts Book, Vol. I:7-20, Academic Press, San Diego CA).

Almost all kinases contain a conserved 250-300 amino acid kinase domain that folds into a

two-lobed structure. The primary structure of the kinase domain is conserved and can be further subdivided into 11 subdomains. The smaller N-terminal lobe of the kinase domain, which contains subdomains I through IV, is primarily involved in the binding and orientation of the ATP (or GTP) donor molecule. The larger C terminal lobe, which contains subdomains VI through XI, binds the protein substrate and carries out transfer of the gamma phosphate from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue. Subdomain V spans the two lobes. Each of the 11 subdomains contains specific amino acid residues and motifs that are characteristic of the particular subdomain and are highly conserved (Hardie, G. and S. Hanks (1995) The Protein Kinase Facts Book, Vol. I:7-20, Academic Press, San Diego CA). In particular, two protein kinase signature sequences have been identified in the kinase domain, the first containing an active site lysine residue involved in ATP binding (subdomain II), and the second containing an aspartate residue important for catalytic activity (subdomain VI). Kinases may also be categorized into families by the different amino acid sequences (generally between 5 and 100 residues) located on either side of, or inserted into, the kinase domain. These additional amino acid sequences are involved in the regulation of each kinase as the kinase recognizes and interacts with its target protein.

PTKs may be classified as either transmembrane or non-transmembrane proteins. Transmembrane PTKs function as receptors for most growth factors. Growth factors bind to the receptor tyrosine kinase (RTK), causing the RTK to phosphorylate itself (autophosphorylation) and specific intracellular second messenger proteins. Growth factors that bind RTKs include epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, hepatocyte growth factor, insulin and insulin-like growth factors, nerve growth factor, vascular endothelial growth factor, and macrophage colony stimulating factor.

Non-transmembrane PTKs form signaling complexes with the cytosolic domains of plasma membrane receptors. Receptors that signal through non-transmembrane PTKs include receptors for cytokines and hormones (e.g., growth hormone and prolactin), and antigen-specific receptors on T and B lymphocytes. Many non-transmembrane PTKs were first identified as the products of mutant oncogenes in cancer cells in which PTK activation was no longer subject to normal cellular controls. About one third of the known oncogenes encode PTKs, and it is well known that cellular transformation (oncogenesis) is often accompanied by increased tyrosine phosphorylation activity (Charbonneau H and N.K. Tonks (1992) Annu. Rev. Cell Biol. 8:463-493). Regulation of PTK activity may therefore be an important strategy in controlling some types of cancer.

STKs are non-transmembrane proteins. STKs include second messenger-dependent protein kinases, which primarily mediate the effects of second messengers such as cyclic AMP, cyclic GMP, inositol triphosphate, phosphatidylinositol 3,4,5-triphosphate, cyclic ADP ribose, arachidonic acid, diacylglycerol, and calcium-calmodulin (CaM). STKs include cyclic AMP dependent protein kinases

(PKAs), which are involved in mediating hormone-induced cellular responses; CaM-dependent protein kinases, which are involved in regulation of smooth muscle contraction, glycogen breakdown, and neurotransmission; mitogen-activated protein (MAP) kinases, which mediate signal transduction from the cell surface to the nucleus via phosphorylation cascades; and diacylglycerol-activated protein kinase C (PKC), which mediates glycogen breakdown and activation of various transcription factors. PKC mu is a novel member of the PKC family that, like other PKCs, contains a zinc-finger-like, cysteine-rich motif in the N-terminal region necessary for phorbol ester binding, and is also capable of phorbol ester-independent kinase activity (Johannes, F.J. et al. (1994) J. Biol. Chem. 269:6140-6148).

The PKAs are activated by cAMP produced within the cell in response to hormone stimulation. Altered PKA expression is implicated in a variety of disorders and diseases including cancer, thyroid disorders, diabetes, atherosclerosis, and cardiovascular disease (Isselbacher, K.J. et al. (1994) Harrison's Principles of Internal Medicine, McGraw-Hill, New York NY, pp. 416-431, 1987). CaM dependent protein kinases are activated by calmodulin, an intracellular calcium receptor, in response to the concentration of free calcium in the cell. CaM kinase I and CaM kinase II play important roles in the regulation of neurotransmission, and kinases have been associated with neurological disorders such as Alzheimer's disease and with cognitive effects of aging. (See, for example, Lynch, M.A. (1998) Prog. Neurobiol. 56:571-589 and Bonkale, W.L. et al. (1999) Brain Res. 818:383-396.) CASK is a neuronal cell surface protein (neurexin) that includes a calmodulin-dependent protein kinase domain and is present in relatively high concentrations in brain synaptic plasma membranes (Hata, Y. et al. (1996) J. Neurosci. 16:2488-2494). CASK forms part of a complex capable of binding the amyloid precursor protein (APP) implicated in Alzheimer's Disease, and may play an important role in APP processing (Borg, J.P. et al. (1998) J. Biol. Chem. 273:31633-31636).

The cyclin-dependent protein kinases (CDKs) are STKs that control the progression of cells through the cell cycle. Cyclins are small regulatory proteins that bind to and activate CDKs, which then phosphorylate and activate selected proteins involved in the mitotic process. CDKs are unique in that they require multiple inputs to become activated. In addition to cyclin, CDK activation requires the phosphorylation of a specific threonine residue and the dephosphorylation of a specific tyrosine residue. Another family of STKs associated with the cell cycle are the NIMA (never in mitosis)-related kinases (Neks). Both CDKs and Neks are involved in duplication, maturation, and separation of the microtubule organizing center, the centrosome, in animal cells (Fry, A.M. et al. (1998) EMBO J. 17:470-481).

The MAP kinases comprise another STK family that regulates intracellular signaling pathways. The MAP kinases mediate signal transduction from the cell surface to the nucleus via

phosphorylation cascades. The extracellular stimuli which activate MAP kinase pathways include epidermal growth factor, ultraviolet light, hyperosmolar medium, heat shock, endotoxic lipopolysaccharide, and pro-inflammatory cytokines such as tumor necrosis factor and interleukin-1. Altered MAP kinase expression is implicated in a variety of disease conditions including cancer, inflammation, immune disorders, and disorders affecting growth and development.

Apoptosis is a highly regulated signaling pathway leading to cell death that plays a crucial role in tissue development and homeostasis. Deregulation of this process is associated with the pathogenesis of a number of diseases including autoimmune disease, neurodegenerative disorders, and cancer. Various STKs play key roles in this process. ZIP kinase is an STK containing a C-terminal leucine zipper domain in addition to its N-terminal protein kinase domain. This C-terminal domain appears to mediate homodimerization and activation of the kinase as well as interactions with transcription factors such as activating transcription factor, ATF4, a member of the cyclic-AMP responsive element binding protein (ATF/CREB) family of transcriptional factors (Sanjo, H. et al. (1998) J. Biol. Chem, 273:29066-29071). DRAK1 and DRAK2 are STKs that share homology with the death-associated protein kinases (DAP kinases), known to function in interferon-γ induced apoptosis (Sanjo et al., supra). Like ZIP kinase, DAP kinases contain a C-terminal protein-protein interaction domain, in the form of ankyrin repeats, in addition to the N-terminal kinase domain. These types of kinases, ZIP, DAP, and DRAK, induce morphological changes associated with apoptosis when transfected into NIH3T3 cells (Sanjo et al., supra). However, deletion of either the N-terminal kinase catalytic domain or the C-terminal domain of these proteins abolishes apoptotic activity, indicating that in addition to the kinase activity, activity in the C-terminal domain is also necessary for apoptosis, possibly as an interacting domain with a regulator or a specific substrate.

RICK is another STK recently identified as mediating a specific apoptotic pathway involving the death receptor, CD95 (Inohara, N. et al. (1998) J. Biol. Chem. 273:12296-12300). CD95 is a member of the tumor necrosis factor receptor superfamily and plays a critical role in the regulation and homeostasis of the immune system (Nagata, S. (1997) Cell 88:355-365). The CD95 receptor signaling pathway involves recruitment of various intracellular molecules to a receptor complex following ligand binding. This includes recruitment of the cysteine protease caspase-8 which, in turn, activates a caspase cascade leading to cell death. RICK is composed of an N-terminal kinase catalytic domain and a C-terminal "caspase-recruitment" domain that interacts with caspase-like domains, indicating that RICK plays a role in the recruitment of caspase-8. This interpretation is supported by the fact that the expression of RICK in human 293T cells promotes activation of caspase-8 and potentiates the induction of apoptosis by various proteins involved in the CD95 apoptotic pathway (Inohara et al., supra).

Protein phosphatases regulate the effects of protein kinases by removing phosphate groups from molecules activated by kinases. Phosphatases are characterized as either tyrosine-specific or serine/threonine-specific based on their preferred phospho-amino acid substrate, although some protein phosphatases have dual specificity for both serine/threonine and tyrosine groups. Serine/threonine phosphatases dephosphorylate phosphoserine and phosphothreonine residues, and are important regulators of many cAMP-mediated hormone responses (Cohen, P. (1989) Annu. Rev. Biochem. 58:453-508). Serine/threonine phosphatases generally comprise two or more subunits and have broad and overlapping protein substrate specificities.

Tyrosine phosphatases are generally monomeric proteins which function primarily in the transduction of signals across the plasma membrane, and are categorized as either transmembrane receptor-like proteins or soluble non-transmembrane proteins. Tyrosine phosphatases reverse the effects of PTKs, removing phosphate groups from tyrosine residues of phosphorylated proteins, and play a significant role in cell cycle and cell signaling processes, lymphocyte activation, and cell adhesion (Charbonneau, H. and N.K. Tonks (1992) Annu. Rev. Cell Biol. 8:463-493). In the process of cell division, for example, a specific tyrosine phosphatase called M-phase inducer phosphatase plays a key role in the induction of mitosis by dephosphorylating and activating CDC2, a cell division-specific PTK (Krishna, S. et al. (1990) Proc. Natl. Acad. Sci. USA 87:5139-5143).

Tyrosine phosphatases share a conserved catalytic domain of about 250 amino acids which contains the active site. The active site consensus sequence consists of 13 amino acids, including a cysteine residue that is essential for phosphatase activity. In addition, the genes encoding at least eight tyrosine phosphatases have been mapped to chromosomal regions that are translocated or rearranged in various neoplastic conditions, including lymphoma, leukemia, small cell lung carcinoma, adenocarcinoma, and neuroblastoma (Charbonneau, H. and N.K. Tonks (1992) Annu. Rev. Cell Biol. 8:463-493). As previously noted, many PTKs are encoded by oncogenes, and oncogenesis is often accompanied by increased tyrosine phosphorylation activity. It is therefore possible that tyrosine phosphatases may prevent or reverse cell transformation and the growth of various cancers by controlling the levels of tyrosine phosphorylation in cells. This hypothesis is supported by studies showing that overexpression of tyrosine phosphatases can suppress transformation in cells, and that specific inhibition of tyrosine phosphatases can enhance cell transformation (Charbonneau and Tonks, <u>supra</u>).

In addition to protein phosphorylation, lipid phosphorylation also plays a role in certain signaling pathways. The phosphorylation of phosphatidylinositol is involved in activation of the PKC signaling pathway. Recently, a sphingolipid metabolite, sphingosine-1-phosphate (SPP), has emerged as a novel lipid second-messenger with both extracellular and intracellular actions (Kohama, T. et al. (1998) J. Biol. Chem. 273:23722-23728). Extracellularly, SPP is a ligand for the G-protein

coupled receptor EDG-1 (endothelial-derived, G-protein coupled receptor). Intracellularly, SPP regulates cell growth, survival, motility, and cytoskeletal changes. SPP levels are regulated by sphingosine kinases that specifically phosphorylate D-erythro-sphingosine to SPP. The importance of sphingosine kinase in cell signaling is indicated by the fact that various stimuli, including platelet-derived growth factor (PDGF), nerve growth factor, and activation of PKC, increase cellular levels of SPP by activation of sphingosine kinase, and the fact that competitive inhibitors of the enzyme selectively inhibit cell proliferation induced by PDGF (Kohama et al., supra).

The discovery of new regulators of intracellular phosphorylation and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of neurological, cell proliferative, and autoimmune/inflammatory disorders.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, regulators of intracellular phosphorylation, referred to collectively as "HRIP" and individually as "HRIP-1," "HRIP-2," "HRIP-3," "HRIP-4," "HRIP-5," "HRIP-6," "HRIP-7," "HRIP-8," "HRIP-9," "HRIP-10," "HRIP-11," "HRIP-12," "HRIP-13," and "HRIP-14." In one aspect, the invention provides an isolated polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:15-28.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected

from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide comprising a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), or d) a polynucleotide sequence complementary to b). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), or d) a polynucleotide sequence complementary to b). The method comprises a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed

between said probe and said target polynucleotide, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 30 contiguous nucleotides. In another alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and a pharmaceutically acceptable excipient. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional HRIP, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional HRIP, comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention

provides a method of treating a disease or condition associated with overexpression of functional HRIP, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:15-28, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HRIP.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HRIP.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HRIP were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze HRIP, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HRIP" refers to the amino acid sequences of substantially purified HRIP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HRIP. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HRIP either by directly interacting with HRIP or by acting on components of the biological pathway in which HRIP participates.

An "allelic variant" is an alternative form of the gene encoding HRIP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HRIP include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HRIP or a polypeptide with at least one functional characteristic of HRIP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HRIP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HRIP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HRIP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HRIP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged

amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of HRIP. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HRIP either by directly interacting with HRIP or by acting on components of the biological pathway in which HRIP participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HRIP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates,

methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HRIP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HRIP or fragments of HRIP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HRIP or the polynucleotide encoding HRIP which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example,

a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:15-28 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:15-28, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:15-28 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:15-28 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:15-28 and the region of SEQ ID NO:15-28 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-14 is encoded by a fragment of SEQ ID NO:15-28. A fragment of SEQ ID NO:1-14 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-14. For example, a fragment of SEQ ID NO:1-14 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-14. The precise length of a fragment of SEQ ID NO:1-14 and the region of SEQ ID NO:1-14 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity

(e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at http://www.ncbi.nlm.nih.gov/BLAST/. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known.. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 μg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present

invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100-200 μg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of HRIP which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of HRIP which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of HRIP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HRIP.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or

synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Probe" refers to nucleic acid sequences encoding HRIP, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al.,1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to

100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose

instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HRIP, or fragments thereof, or HRIP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor

of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of th present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human regulators of intracellular phosphorylation (HRIP), the polynucleotides encoding HRIP, and the use of these compositions for

the diagnosis, treatment, or prevention of neurological, cell proliferative, and autoimmune/inflammatory disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HRIP. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each HRIP were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. The Incyte clones in column 5 were used to assemble the consensus nucleotide sequence of each HRIP and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HRIP. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:15-28 and to distinguish between SEQ ID NO:15-28 and related polynucleotide sequences. For SEQ ID NO:15-27, the polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HRIP as a fraction of total tissues expressing HRIP. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HRIP as a fraction of total tissues expressing HRIP. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding HRIP were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

The invention also encompasses HRIP variants. A preferred HRIP variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the HRIP amino acid sequence, and which contains at least one functional or structural

characteristic of HRIP.

The invention also encompasses polynucleotides which encode HRIP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28, which encodes HRIP. The polynucleotide sequences of SEQ ID NO:15-28, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding HRIP. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HRIP. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28 which has at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:15-28. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HRIP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HRIP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HRIP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HRIP and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HRIP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HRIP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HRIP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HRIP and

HRIP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HRIP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:15-28 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HRIP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme

digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HRIP may be cloned in recombinant DNA molecules that direct expression of HRIP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HRIP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HRIP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction

sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of HRIP, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding HRIP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, HRIP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HRIP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HRIP, the nucleotide sequences encoding HRIP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and

inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HRIP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HRIP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HRIP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HRIP and appropriate transcriptional and translational control elements. These methods include <u>in vitro</u> recombinant DNA techniques, synthetic techniques, and <u>in vivo</u> genetic recombination. (See, e.g., Sambrook, J. et al. (1989) <u>Molecular Cloning, A Laboratory Manual</u>, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HRIP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HRIP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HRIP can be achieved using a multifunctional <u>E. coli</u> vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HRIP into the vector's multiple cloning site disrupts the *lac*Z gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for <u>in vitro</u> transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HRIP are needed, e.g. for the production of

antibodies, vectors which direct high level expression of HRIP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HRIP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HRIP. Transcription of sequences encoding HRIP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HRIP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HRIP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HRIP in cell lines is preferred. For example, sequences encoding HRIP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the

introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*⁻ and *apr*⁻ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), ß glucuronidase and its substrate ß-glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HRIP is inserted within a marker gene sequence, transformed cells containing sequences encoding HRIP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HRIP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HRIP and that express HRIP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HRIP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and

fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HRIP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HRIP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HRIP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HRIP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HRIP may be designed to contain signal sequences which direct secretion of HRIP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid

sequences encoding HRIP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HRIP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HRIP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HRIP encoding sequence and the heterologous protein sequence, so that HRIP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HRIP may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ^{35}S-methionine.

Fragments of HRIP may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HRIP may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HRIP and regulators of intracellular phosphorylation. In addition, the expression of HRIP is closely associated with neurological tissue, with cancer and other cell proliferative disorders, and with inflammation and the immune response. Therefore, HRIP appears to play a role in neurological, cell proliferative, and autoimmune/inflammatory disorders. In the treatment of disorders associated with increased HRIP expression or activity, it is desirable to decrease the expression or activity of HRIP. In the treatment of disorders associated with decreased HRIP

expression or activity, it is desirable to increase the expression or activity of HRIP.

Therefore, in one embodiment, HRIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HRIP. Examples of such disorders include, but are not limited to, a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and a cancer including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's

thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocyt penic purpura, ulcerative c litis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extrac rporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

In another embodiment, a vector capable of expressing HRIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HRIP including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HRIP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HRIP including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HRIP may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HRIP including, but not limited to, those listed above.

In a further embodiment, an antagonist of HRIP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HRIP. Examples of such disorders include, but are not limited to, those neurological, cell proliferative, and autoimmune/inflammatory disorders described above. In one aspect, an antibody which specifically binds HRIP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HRIP.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HRIP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HRIP including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HRIP may be produced using methods which are generally known in the art. In particular, purified HRIP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HRIP. Antibodies to HRIP may also

be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HRIP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HRIP have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HRIP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HRIP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HRIP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as

disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HRIP may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HRIP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HRIP epitopes is generally used, but a competitive binding assay may also be employed (Pound, <u>supra</u>).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HRIP. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of HRIP-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HRIP epitopes, represents the average affinity, or avidity, of the antibodies for HRIP. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular HRIP epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the HRIP-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HRIP, preferably in active form, from the antibody (Catty, D. (1988) <u>Antibodies, Volume I: A Practical Approach</u>, IRL Press, Washington, DC; Liddell, J.E. and Cryer, A. (1991) <u>A Practical Guide to Monoclonal Antibodies</u>, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HRIP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and

guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding HRIP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HRIP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HRIP. Thus, complementary molecules or fragments may be used to modulate HRIP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HRIP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HRIP. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding HRIP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HRIP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HRIP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme

molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HRIP.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HRIP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HRIP, antibodies to HRIP, and mimetics, agonists, antagonists, or inhibitors of HRIP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HRIP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs.

An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HRIP or fragments thereof, antibodies of HRIP, and agonists, antagonists or inhibitors of HRIP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HRIP may be used for the diagnosis of disorders characterized by expression of HRIP, or in assays to monitor patients being treated with HRIP or agonists, antagonists, or inhibitors of HRIP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HRIP include methods which utilize the antibody and a label to detect HRIP in human body fluids

or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HRIP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HRIP expression. Normal or standard values for HRIP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HRIP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HRIP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HRIP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HRIP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HRIP, and to monitor regulation of HRIP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HRIP or closely related molecules may be used to identify nucleic acid sequences which encode HRIP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HRIP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HRIP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:15-28 or from genomic sequences including promoters, enhancers, and introns of the HRIP gene.

Means for producing specific hybridization probes for DNAs encoding HRIP include the cloning of polynucleotide sequences encoding HRIP or HRIP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HRIP may be used for the diagnosis of disorders associated with expression of HRIP. Examples of such disorders include, but are not limited to, a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and a cancer including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis,

polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Wern r syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma. The polynucleotide sequences encoding HRIP may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HRIP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HRIP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HRIP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HRIP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HRIP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HRIP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance

of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HRIP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HRIP, or a fragment of a polynucleotide complementary to the polynucleotide encoding HRIP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of HRIP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HRIP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HRIP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HRIP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HRIP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HRIP, or fragments thereof, and washed. Bound HRIP is then detected by methods well known in the art. Purified HRIP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HRIP specifically compete with a test compound for binding HRIP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more

antigenic determinants with HRIP.

In additional embodiments, the nucleotide sequences which encode HRIP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/125,593, U.S. Ser. No. 60/135,049, and U.S. Ser. No. 60/143,188, are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column

chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent E. coli cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed

using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:15-28. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, <u>supra</u>, ch. 7; Ausubel,

1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HRIP occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Extension of HRIP Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:15-28 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction

mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+}, $(NH_4)_2SO_4$, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 μl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 μl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethysulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:15-28 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

VI. Labeling and Us of Individual Hybridizati n Probes

Hybridization probes derived from SEQ ID NO:15-28 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or

selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the HRIP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HRIP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HRIP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HRIP-encoding transcript.

IX. Expression of HRIP

Expression and purification of HRIP is achieved using bacterial or virus-based expression systems. For expression of HRIP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HRIP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HRIP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HRIP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HRIP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step,

affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathi ne under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HRIP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified HRIP obtained by these methods can be used directly in the following activity assay.

X. Demonstration of HRIP Activity

Kinase activity of HRIP is measured by the phosphorylation of appropriate substrates using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a beta radioisotope counter. HRIP is incubated with the protein substrate, ^{32}P-ATP, and an appropriate kinase buffer. The ^{32}P incorporated into the product is separated from free ^{32}P-ATP by electrophoresis and the incorporated ^{32}P is counted. The amount of ^{32}P recovered is proportional to the kinase activity of HRIP in the assay. A determination of the specific amino acid residue phosphorylated by protein kinase activity is made by phosphoamino acid analysis of the hydrolyzed protein.

Alternatively, protein phosphatase activity of HRIP is measured by the hydrolysis of P-nitrophenyl phosphate (PNPP). HRIP is incubated together with PNPP in HEPES buffer pH 7.5, in the presence of 0.1% b-mercaptoethanol at 37°C for 60 min. The reaction is stopped by the addition of 6 ml of 10 N NaOH and the increase in light absorbance at 410 nm resulting from the hydrolysis of PNPP is measured using a spectrophotometer. The increase in light absorbance is proportional to the activity of HRIP in the assay (Diamond, R.H. et al. (1994) Mol. Cell Biol. 14:3752-3762).

XI. Functional Assays

HRIP function is assessed by expressing the sequences encoding HRIP at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP;

Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HRIP on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HRIP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HRIP and other genes of interest can be analyzed by northern analysis or microarray techniques.

XII. Production of HRIP Specific Antibodies

HRIP substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HRIP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HRIP activity by, for example, binding the peptide or HRIP to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring HRIP Using Specific Antibodies

Naturally occurring or recombinant HRIP is substantially purified by immunoaffinity chromatography using antibodies specific for HRIP. An immunoaffinity column is c nstructed by covalently coupling anti-HRIP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HRIP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HRIP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HRIP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HRIP is collected.

XIV. Identification of Molecules Which Interact with HRIP

HRIP, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HRIP, washed, and any wells with labeled HRIP complex are assayed. Data obtained using different concentrations of HRIP are used to calculate values for the number, affinity, and association of HRIP with the candidate molecules.

Alternatively, molecules interacting with HRIP are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	15	480457	LIVRBCT01	154793R6 (THP1PLB02), 480457H1 (LIVRBCT01), 480457X12R1 (LIVRBCT01), 480457X13R1 (LIVRBCT01), 1817968F6 (PROSNOT20), 3149829R6 (ADRENON04), 3766714H1 (BRSTNOT24)
2	16	563663	NEUTLPT01	285464X4 (EOSIHET02), 285464X8 (EOSIHET02), 563663H1 (NEUTLPT01)
3	17	1425842	BEPINON01	120376R6 (MUSCNOT01), 1425842H1 (BEPINON01), 1571293F1 (UTRSNOT05), 1851503F6 (LUNGFET03), 3596860F6 (FIBPNOT01)
4	18	2349047	COLSUCT01	1718442F6 (BLADNOT06), 1960909R6 (BRSTNOT04), 1960909T6 (BRSTNOT04), 2349047H1 (COLSUCT01), SBHA02478F1, SBHA02364F1, SBHA00749F1
5	19	2415617	HNT3AZT01	471426R6 (MMLR1DT01), 941244R1 (ADRENOT03), 1466986F1 (PANCTUT02), 1519153H1 (BLADTUT04), 2301362R6 (BRSTNOT05), 2415617H1 (HNT3AZT01), 2482778F6 (SMCANOT01), 4410796H1 (MONOTXT01)
6	20	3815186	TONSNOT03	1759852R3 (PITUNOT03), 3815186H1 (TONSNOT03), SBJA02796F1
7	21	5504544	BRABDIR01	046651X31 (CORNNOT01), 179527X7 (PLACNOB01), 905771T1 (COLNNOT08), 1291784F6 (PGANNOT03), 1291784T6 (PGANNOT03), 1542946X14 (PROSTUT04), 2759765R6 (THP1AZS08), 2908366F6 (THYMNOT05), 5504544H1 (BRABDIR01)
8	22	1511326	LUNGNOT14	1511326H1 (LUNGNOT14), 1511326F1 (LUNGNOT14), 1511326T1 (LUNGNOT14), 1511326T6 (LUNGNOT14), 2922438F6 (SININOT04), 3029015H1 (HEARFET02), 5167787H1 (MUSCDMT01)
9	23	1519120	BLADTUT04	1519120H1 (BLADTUT04), 1519120F6 (BLADTUT04), 1519120T6 (BLADTUT04)

Table 1 (cont.)

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
10	24	1673761	BLADNOT05	1673761H1 (BLADNOT05), 1673761F6 (BLADNOT05), 1673761T6 (BLADNOT05), 2024815R6 (KERANOT02), 2111282H1 (BRAITUT03), 2655266F6 (THYMNOT04), 4178671H1 (BRAINOT22), 5307169H1 (MONOTXT02), 1424569T1 (BEPINON01)
11	25	1270442	BRAINOT09	401269R6 (TMLR3DT01), 1270442H1 (BRAINOT09), 1672906F6 (BLADNOT05), 2596974H1 (OVARTUT02), 2794801H1 (NPOLNOT01), 3289454H1 (BONRFET01), 3293681H1 (TLYJINT01), 3377437H1 (PENGNOT01), 3744502H1 (THYMNOT08)
12	26	1877133	LEUKNOT03	076555H1 (THP1PEB01), 1422142F6 (KIDNNOT09), 1877133H1 (LEUKNOT03), 2403383F6 (SMCANOT01), 2483377H1 (SMCANOT01)
13	27	2636759	BONTNOT01	2636759F6 (BONTNOT01), 2636759H1 (BONTNOT01), SBUA02427D1
14	28	2716815	THYRNOT09	078565R1 (SYNORAB01), 079174R1 (SYNORAB01), 1272190X14 (TESTTUT02), 1272190X23R1 (TESTTUT02), 1272190X24R1 (TESTTUT02), 2680827H1 (SINIUCT01), 2716815H1 (THYRNOT09), 2716815T6 (THYRNOT09), 3272956H1 (PROSBPT06)

Table 2

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Homologous Sequence	Analytical Methods
1	482	T160 S4 T211 S217 T313 T430 S52 S67 T140 S146 S201 S217 S224 S258 S275 S378 T467 T472	N57 N184 N353	Dual-specificity phosphatase catalytic site: E321-D461	Dual-specificity protein tyrosine phosphatase [Rattus norvegicus] (g1185552)	BLAST MOTIFS PFAM BLOCKS PRINTS
2	190	T35 T55 S131 S2 S183 Y147	N102	Protein kinase ATP-binding site: L39-K62 Protein kinase catalytic site: I154-L166 Eukaroytic protein kinase domain: I33-I186	DRAK2 kinase [Homo sapiens] (g3834356)	BLAST MOTIFS PFAM PRINTS
3	455	S252 S89 S234 S258 S268 S302 S342 S346 S364 S429 S434 S61 S96 S302 S410 T414 S415 Y343	N97 N159 N265 N409	Protein kinase ATP-binding site: V129-I141 Eukaroytic protein kinase domain: L16-I257 Leucine zipper: L294-L322	Serine/threonine protein kinase ZIP [Homo sapiens] (g561543)	BLAST MOTIFS PFAM PRINTS
4	485	S166 S283 S16 T167 S208 S242 T267 S283 T292 S306 T354 S278 T336 T370 S402 T412 S449 S483	N66 N400 N421 N481	Protein kinase catalytic site: L105 -L117 Eukaroytic protein kinase domain: R26-L247	Serine/threonine kinase RICK [Homo sapiens] (g3123887)	BLAST MOTIFS PRINTS PFAM
5	384	T130 T54 S181 T205 S371	N137	Diacylglycerol kinase catalytic domain: R16-L153	Sphingosine kinase [Mus musculus] (g3659694)	BLAST PFAM BLOCKS

Table 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Homologous Sequence	Analytical Methods
6	81	T37 S38 T52 T73 T54		Protein kinase C terminal domain: D33-F77	Protein kinase [Rattus norvegicus] (g206191)	BLAST PFAM
7	721	S40 S239 S640 T61 S68 S176 S196 S205 T241 S251 T416 T432 T655 T696 S49 T90 S230 T234 T235 S251 T255 T277 T416 S447 S484 S549 T696	N30 N274 N275 N297 N316 N572	Protein kinase ATP-binding site: L400-K423 Protein kinase catalytic site: I513-L525 Eukaroytic protein kinase domain: I394-L650 Phorbol ester/ diacylglycerol binding domain: H108-C157	Protein kinase C mu [Homo sapiens] (g438373)	BLAST MOTIFS PFAM PRINTS
8	249	S3 S4 T38 T137 S150 T64 T75 S107 S119 S196	N204	Tyrosine specific protein phosphatases active site: V88-I100 Tyrosine specific protein phosphatase: V88-S98	Putative tyrosine phosphatase [Homo sapiens] (g6650693)	MOTIFS BLOCKS BLAST
9	146	S125 S131		Eukaryotic protein kinase domain: Y12-L105	mCASK-A [Mus musculus] (g3087816)	MOTIFS BLAST PFAM
10	524	T21 T31 S77 S190 S237 S311 S511 S198 S207 T417 S440	N189	Eukaryotic protein kinase domain: P247-P492 Protein kinase signatur s: L253-K276, L368-L380	Protein kinase homolog [Arabidopsis thaliana] (g2244835)	MOTIFS BLAST PFAM PRINTS PROFILESCAN

Table 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Homologous Sequence	Analytical Methods
11	509	S224 T45 S80 T95 T216 T262 S307 S317 T325 S343 T388 S434 T452 S466 S7 S212 S282	N208	Protein phosphatase pp2a regulatory subunit, alternative splicing: S56-E462 Protein phosphatase pp2a regulatory subunit: S56-V312 EF-hand calcium-binding domain: D335-L347	Protein phosphatase 2A 72 kDa regulatory subunit [Homo sapiens] (g190222)	MOTIFS BLAST-GenBank BLAST-PRODOM BLAST-DOMO
12	142	S18 T26 T77 T4 T116	N114		TAK1 TGF-beta activated kinase [Xenopus laevis] (g3057036)	MOTIFS BLAST-GenBank
13	221	S21 S208 T2 T69 T170 S9 S16		Tyrosine specific protein phosphatase active site: V146-L158 Dual specificity protein tyrosine phosphatase: D121-L201 VH1-type dual specificity phosphatase: E57-V202	Tyrosine/serine phosphatase [Homo sapiens] (g181840)	MOTIFS BLAST-GenBank BLAST-PRODOM BLAST-DOMO HMMER-PFAM PROFILESCAN
14	462	T119 S302 S390 S36 S59 S163 T167 T175 S177 T187 S218 S276 T309 S311 S434 T443 S35 S87 S200 T242 S334 S360 T436	N57	Tyrosine kinase: L139-P427	Multihormonally regulated gene [Rattus norvegicus] (g1561667)	MOTIFS BLAST-GenBank BLAST-DOMO

Table 3

Polynucleotide SEQ ID NO:	Useful Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
15	435-479	Reproductive (0.300) Cardiovascular (0.150) Hematopoietic/Immune (0.150)	Cell Proliferation (0.450) Inflammation (0.500)	PBLUESCRIPT
16	219-263	Hematopoietic/Immune (0.455) Cardiovascular (0.182) Developmental (0.091)	Cell Proliferation (0.546) Inflammation (0.455)	PBLUESCRIPT
17	1191-1235	Reproductive (0.250) Cardiovascular (0.214) Gastrointestinal (0.214)	Cell Proliferation (0.465) Inflammation (0.322)	pT7T3
18	542-586 974-1018	Gastrointestinal (0.391) Hematopoietic/Immune (0.174) Reproductive (0.174)	Inflammation (0.478) Cell Proliferation (0.391)	pINCY
19	217-261 718-761	Cardiovascular (0.256) Reproductive (0.179) Hematopoietic/Immune (0.154)	Cell Proliferation (0.564) Inflammation (0.334)	pINCY
20	380-424	Nervous (0.520) Hematopoietic/Immune (0.240) Reproductive (0.120)	Inflammation (0.520) Cell Proliferation (0.400)	pINCY
21	487-531 1027-1071	Reproductive (0.296) Hematopoietic/Immune (0.198) Gastrointestinal (0.111)	Cell Proliferation (0.568) Inflammation (0.358)	pINCY
22	379-423	Cardiovascular (0.267) Gastrointestinal (0.200) Hematopoietic/Immune (0.200)	Inflammation (0.333) Cancer (0.267) Trauma (0.200)	pINCY
23	192-218	Nervous (0.500) Urologic (0.500)	Cancer (0.500) Cell Line (0.500)	pINCY

Table 3 (cont.)

Polynucleotide SEQ ID NO:	Useful Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
24	163-207	Nervous (0.333) Cardiovascular (0.133) Dermatologic (0.133)	Cancer (0.400) Fetal/Cell Line (0.200) Inflammation (0.133)	pINCY
25	19-63	Cardiovascular (0.182) Hematopoietic/Immune (0.182) Reproductive (0.182)	Cancer (0.515) Cell Proliferation (0.242) Inflammation (0.242)	pINCY
26	297-343	Cardiovascular (0.250) Hematopoietic/Immune (0.150) Musculoskeletal (0.150)	Cancer (0.300) Cell Proliferation (0.250) Inflammation (0.200)	pINCY
27	271-315	Endocrine (0.500) Musculoskeletal (0.500)	Cancer (0.500)	pINCY
28	161-207	Reproductive (0.241) Gastrointestinal (0.233) Cardiovascular (0.150)	Cancer (0.429) Inflammation (0.263) Cell Proliferation (0.211)	pINCY

Table 4

Polynucleotide SEQ ID NO:	Library	Library Comment
15	LIVRBCT01	Library was constructed using RNA isolated from the liver tissue of a patient with primary biliary cirrhosis who had a liver transplant.
16	NEUTLPT01	Library was constructed using RNA isolated from peripheral blood granulocytes collected by density gradient centrifugation through Ficoll-Hypaque. The cells were isolated from buffy coat units obtained from unrelated male and female donors. Cells were cultured in 100 ng/ml E. coli LPS for 30 minutes.
17	BEPINON01	This normalized bronchial epithelium library was constructed from 5.12 million independent clones from a bronchial epithelium library. RNA was made from a bronchial epithelium primary cell line derived from a 54-year-old Caucasian male. The normalization and hybridization conditions were adapted from Soares et al., Proc. Natl. Acad. Sci. USA (1994) 91:9228, using a longer (24-hour) reannealing hybridization period.
18	COLSUCT01	Library was constructed using RNA isolated from diseased sigmoid colon tissu obtained from a 70-year-old Caucasian male during colectomy with permanent ileostomy. Pathology indicated chronic ulcerative colitis. Patient history included benign neoplasm of the colon. Family history included atherosclerotic coronary artery disease and myocardial infarctions.
19	HNT3AZT01	Library was constructed using RNA isolated from the hNT2 cell line (derived from a human teratocarcinoma that exhibited properties characteristic of a committed neuronal precursor). Cells were treated for three days with 0.35 micromolar 5-aza-2'-deoxycytidine (AZ).
20	TONSNOT03	Library was constructed using RNA isolated from diseased left tonsil tissue removed from a 6-year-old Caucasian male during adenotonsillectomy. Pathology indicated reactive lymphoid hyperplasia, bilaterally. Family history included benign hypertension, myocardial infarction, and atherosclerotic coronary artery disease.
21	BRABDIR01	Library was constructed using RNA isolated from diseased cerebellum tissue removed from the brain of a 57-year-old Caucasian male, who died from a cerebrovascular accident.
22	LUNGNOT14	Library was constructed using RNA isolated from lung tissue removed from the 1 ft lower lobe of a 47-year-old Caucasian male during a segmental lung resection. Pathology for the associated tumor tissue indicated a grade 4 adenocarcinoma, and the parenchyma showed calcified granuloma. Patient history included benign hypertension and chronic obstructive pulmonary disease. Family history included type II diabetes and acute myocardial infarction.

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Library	Library Comment
23	BLADTUT04	Library was constructed using RNA isolated from bladder tumor tissue removed from a 60-year-old Caucasian male during a radical cystectomy, prostatectomy, and vasectomy. Pathology indicated grade 3 transitional cell carcinoma in the left bladder wall. Carcinoma in-situ was identified in the dome and trigone. Family history included type I diabetes, malignant neoplasm of the stomach, atherosclerotic coronary artery disease, and acute myocardial infarction.
24	BLADNOT05	Library was constructed using RNA isolated from bladder tissue removed from a 60-year-old Caucasian male during a radical cystectomy, prostatectomy, and vasectomy. Pathology for the associated tumor tissue indicated grade 3 transitional cell carcinoma. Carcinoma in-situ was identified in the dome and trigone. Family history included type I diabetes, malignant neoplasm of the stomach, atherosclerotic coronary artery disease, and acute myocardial infarction.
25	BRAINOT09	Library was constructed using RNA isolated from brain tissue removed from a Caucasian male fetus, who died at 23 weeks' gestation.
26	LEUKNOT03	Library was constructed using RNA isolated from white blood cells of a 27-year-old female with blood type A+. The donor tested negative for cytomegalovirus (CMV).
27	BONTNOT01	Library was constructed using RNA isolated from tibial periosteum removed from a 20-year-old Caucasian male during a hemipelvectomy with amputation above the knee. Pathology for the associated tumor tissue indicated partially necrotic and cystic osteoblastic grade 3 osteosarcoma (post-chemotherapy). Family history included osteogenesis imperfecta, closed fracture, and type II diabetes.
28	THYRNOT09	Library was constructed using RNA isolated from diseased thyroid tissue removed from an 18-year-old Caucasian female during an unilateral thyroid lobectomy and regional lymph node excision. Pathology indicated adenomatous goiter. This was associated with a follicular adenoma of the thyroid. Family history included thyroid cancer.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	*ESTs:* Probability value= 1.0E-8 or less *Full Length sequences:* Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	*ESTs:* fasta E value=1.06E-6 *Assembled ESTs:* fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less *Full Length sequences:* fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score≥GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. supra; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a) an amino acid sequ nce selected from the group consisting f SEQ ID NO:1-14,

b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14,

c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and

d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.

2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-14.

3. An isolated polynucleotide encoding a polypeptide of claim 1.

4. An isolated polynucleotide of claim 3 selected from the group consisting of SEQ ID NO:15-28.

5. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.

6. A cell transformed with a recombinant polynucleotide of claim 5.

7. A transgenic organism comprising a recombinant polynucleotide of claim 5.

8. A method for producing a polypeptide of claim 1, the method comprising:

a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and

b) recovering the polypeptide so expressed.

9. An isolated antibody which specifically binds to a polypeptide of claim 1.

10. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

a) a polynucle tide sequence selected from the group consisting of SEQ ID NO:15-28,

b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28,

c) a polynucleotide sequence complementary to a),

d) a polynucleotide sequence complementary to b), and

e) an RNA equivalent of a)-d).

11. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 10.

12. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 10, the method comprising:

a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

13. A method of claim 12, wherein the probe comprises at least 30 contiguous nucleotides.

14. A method of claim 12, wherein the probe comprises at least 60 contiguous nucleotides.

15. A pharmaceutical composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

16. A method for treating a disease or condition associated with decreased expression of functional HRIP, comprising administering to a patient in need of such treatment the pharmaceutical composition of claim 15.

17. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting agonist activity in the sample.

18. A pharmaceutical comp sition comprising an agonist compound identified by a method of claim 17 and a pharmaceutically acceptable excipient.

19. A method for treating a disease or condition associated with decreased expression of functional HRIP, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 18.

20. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting antagonist activity in the sample.

21. A pharmaceutical composition comprising an antagonist compound identified by a method of claim 20 and a pharmaceutically acceptable excipient.

22. A method for treating a disease or condition associated with overexpression of functional HRIP, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 21.

23. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 4, the method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, and

b) detecting altered expression of the target polynucleotide.

PF-0683 PCT

SEQUENCE LISTING

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BANDMAN, Olga
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YUE,Henry
HILLMAN, Jennifer L.
BAUGHN, Mariah R.
AZIMZAI, Yalda
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Asp Phe Gly Ala Ser Arg Phe His Asn His Thr Thr His Met Ser
                155                 160                 165
Leu Val Gly Thr Phe Pro Trp Met Ala Pro Glu Val Ile Gln Ser
                170                 175                 180
Leu Pro Val Ser Glu Thr Cys Asp Thr Tyr Ser Tyr Gly Val Val
                185                 190                 195
Leu Trp Glu Met Leu Thr Arg Glu Val Pro Phe Lys Gly Leu Glu
                200                 205                 210
Gly Leu Gln Val Ala Trp Leu Val Val Glu Lys Asn Glu Arg Leu
                215                 220                 225
Thr Ile Pro Ser Ser Cys Pro Arg Ser Phe Ala Glu Leu Leu His
```

230 235 240
Gln Cys Trp Glu Ala Asp Ala Lys Lys Arg Pro Ser Phe Lys Gln
245 250 255
Ile Ile Ser Ile Leu Glu Ser Met Ser Asn Asp Thr Ser Leu Pro
260 265 270
Asp Lys Cys Asn Ser Phe Leu His Asn Lys Ala Glu Trp Arg Cys
275 280 285
Glu Ile Glu Ala Thr Leu Glu Arg Leu Lys Lys Leu Glu Arg Asp
290 295 300
Leu Ser Phe Lys Glu Gln Glu Leu Lys Glu Arg Glu Arg Arg Leu
305 310 315
Lys Met Trp Glu Gln Lys Leu Thr Glu Gln Ser Asn Thr Pro Leu
320 325 330
Leu Leu Pro Leu Ala Ala Arg Met Ser Glu Glu Ser Tyr Phe Glu
335 340 345
Ser Lys Thr Glu Glu Ser Asn Ser Ala Glu Met Ser Cys Gln Ile
350 355 360
Thr Ala Thr Ser Asn Gly Glu Gly His Gly Met Asn Pro Ser Leu
365 370 375
Gln Ala Met Met Leu Met Gly Phe Gly Asp Ile Phe Ser Met Asn
380 385 390
Lys Ala Gly Ala Val Met His Ser Gly Met Gln Ile Asn Met Gln
395 400 405
Ala Lys Gln Asn Ser Ser Lys Thr Thr Ser Lys Arg Arg Gly Lys
410 415 420
Lys Val Asn Met Ala Leu Gly Phe Ser Asp Phe Asp Leu Ser Glu
425 430 435
Gly Asp Asp Asp Asp Asp Asp Asp Gly Glu Glu Glu Asp Asn Asp
440 445 450
Met Asp Asn Ser Glu
455

<210> 4
<211> 485
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2349047CD1

<400> 4
Met Ala Lys Gly Gly Ile Phe Pro Arg Pro Arg Cys Asp Ser Ser
1 5 10 15
Ser Leu Leu Glu Cys Arg Lys Ala Ile Ser Arg Glu Val Lys Ala
20 25 30
Met Ala Ser Leu Asp Asn Glu Phe Val Leu Arg Leu Glu Gly Val
35 40 45
Ile Glu Lys Val Asn Trp Asp Gln Asp Pro Lys Pro Ala Leu Val
50 55 60
Thr Lys Phe Met Glu Asn Gly Ser Leu Ser Gly Leu Leu Gln Ser
65 70 75
Gln Cys Pro Arg Pro Trp Pro Leu Leu Cys Arg Leu Leu Lys Glu
80 85 90
Val Val Leu Gly Met Phe Tyr Leu His Asp Gln Asn Pro Val Leu
95 100 105
Leu His Arg Asp Leu Lys Pro Ser Asn Val Leu Leu Asp Pro Glu
110 115 120
Leu His Val Lys Leu Ala Asp Phe Gly Leu Ser Thr Phe Gln Gly
125 130 135

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```
Gly Ser Gln Ser Gly Thr Gly Ser Gly Glu Pro Gly Gly Thr Leu
                140                 145                 150
Gly Tyr Leu Ala Pro Glu Leu Phe Val Asn Val Asn Arg Lys Ala
                155                 160                 165
Ser Thr Ala Ser Asp Val Tyr Ser Phe Gly Ile Leu Met Trp Ala
                170                 175                 180
Val Leu Ala Gly Arg Glu Val Glu Leu Pro Thr Glu Pro Ser Leu
                185                 190                 195
Val Tyr Glu Ala Val Cys Asn Arg Gln Asn Arg Pro Ser Leu Ala
                200                 205                 210
Glu Leu Pro Gln Ala Gly Pro Glu Thr Pro Gly Leu Glu Gly Leu
                215                 220                 225
Lys Glu Leu Met Gln Leu Cys Trp Ser Ser Glu Pro Lys Asp Arg
                230                 235                 240
Pro Ser Phe Gln Glu Cys Leu Pro Lys Thr Asp Glu Val Phe Gln
                245                 250                 255
Met Val Glu Asn Asn Met Asn Ala Ala Val Ser Thr Val Lys Asp
                260                 265                 270
Phe Leu Ser Gln Leu Arg Ser Ser Asn Arg Arg Phe Ser Ile Pro
                275                 280                 285
Glu Ser Gly Gln Gly Gly Thr Glu Met Asp Gly Phe Arg Arg Thr
                290                 295                 300
Ile Glu Asn Gln His Ser Arg Asn Asp Val Met Val Ser Glu Trp
                305                 310                 315
Leu Asn Lys Leu Asn Leu Glu Glu Pro Pro Ser Ser Val Pro Lys
                320                 325                 330
Lys Cys Pro Ser Leu Thr Lys Arg Ser Arg Ala Gln Glu Glu Gln
                335                 340                 345
Val Pro Gln Ala Trp Thr Ala Gly Thr Ser Ser Asp Ser Met Ala
                350                 355                 360
Gln Pro Pro Gln Thr Pro Glu Thr Ser Thr Phe Arg Asn Gln Met
                365                 370                 375
Pro Ser Pro Thr Ser Thr Gly Thr Pro Ser Pro Gly Pro Arg Gly
                380                 385                 390
Asn Gln Gly Ala Glu Arg Gln Gly Met Asn Trp Ser Cys Arg Thr
                395                 400                 405
Pro Glu Pro Asn Pro Val Thr Gly Arg Pro Leu Val Asn Ile Tyr
                410                 415                 420
Asn Cys Ser Gly Val Gln Val Gly Asp Asn Asn Tyr Leu Thr Met
                425                 430                 435
Gln Gln Thr Thr Ala Leu Pro Thr Trp Gly Leu Ala Pro Ser Gly
                440                 445                 450
Lys Gly Arg Gly Leu Gln His Pro Pro Pro Val Gly Ser Gln Glu
                455                 460                 465
Gly Pro Lys Asp Pro Glu Ala Trp Ser Arg Pro Gln Gly Trp Tyr
                470                 475                 480
Asn His Ser Gly Lys
                485
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<210> 5
<211> 384
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2415617CD1

<400> 5
Met Asp Pro Ala Gly Gly Pro Arg Gly Val Leu Pro Arg Pro Cys
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```
 1               5                  10                  15
Arg Val Leu Val Leu Leu Asn Pro Arg Gly Gly Lys Gly Lys Ala
                20                  25                  30
Leu Gln Leu Phe Arg Ser His Val Gln Pro Leu Leu Ala Glu Ala
                35                  40                  45
Glu Ile Ser Phe Thr Leu Met Leu Thr Glu Arg Arg Asn His Ala
                50                  55                  60
Arg Glu Leu Val Arg Ser Glu Glu Leu Gly Arg Trp Asp Ala Leu
                65                  70                  75
Val Val Met Ser Gly Asp Gly Leu Met His Glu Val Val Asn Gly
                80                  85                  90
Leu Met Glu Arg Pro Asp Trp Glu Thr Ala Ile Gln Lys Pro Leu
                95                  100                 105
Cys Ser Leu Pro Ala Gly Ser Gly Asn Ala Leu Ala Ala Ser Leu
                110                 115                 120
Asn His Tyr Ala Gly Tyr Glu Gln Val Thr Asn Glu Asp Leu Leu
                125                 130                 135
Thr Asn Cys Thr Leu Leu Leu Cys Arg Arg Leu Leu Ser Pro Met
                140                 145                 150
Asn Leu Leu Ser Leu His Thr Ala Ser Gly Leu Arg Leu Phe Ser
                155                 160                 165
Val Leu Ser Leu Ala Trp Gly Phe Ile Ala Asp Val Asp Leu Glu
                170                 175                 180
Ser Glu Lys Tyr Arg Leu Gly Glu Met Arg Phe Thr Leu Gly
                185                 190                 195
Thr Phe Leu Arg Leu Ala Ala Leu Arg Thr Tyr Arg Gly Arg Leu
                200                 205                 210
Ala Tyr Leu Pro Val Gly Arg Val Gly Ser Lys Thr Pro Ala Ser
                215                 220                 225
Pro Val Val Val Gln Gln Gly Pro Val Asp Ala His Leu Val Pro
                230                 235                 240
Leu Glu Glu Pro Val Pro Ser His Trp Thr Val Val Pro Asp Glu
                245                 250                 255
Asp Phe Val Leu Val Leu Ala Leu Leu His Ser His Leu Gly Ser
                260                 265                 270
Glu Met Phe Ala Ala Pro Met Gly Arg Cys Ala Ala Gly Val Met
                275                 280                 285
His Leu Phe Tyr Val Arg Ala Gly Val Ser Arg Ala Met Leu Leu
                290                 295                 300
Arg Leu Phe Leu Ala Met Glu Lys Gly Arg His Met Glu Tyr Glu
                305                 310                 315
Cys Pro Tyr Leu Val Tyr Val Pro Val Val Ala Phe Arg Leu Glu
                320                 325                 330
Pro Lys Asp Gly Lys Gly Val Phe Ala Val Asp Gly Glu Leu Met
                335                 340                 345
Val Ser Glu Ala Val Gln Gly Gln Val His Pro Asn Tyr Phe Trp
                350                 355                 360
Met Val Ser Gly Cys Val Glu Pro Pro Pro Ser Trp Lys Pro Gln
                365                 370                 375
Gln Met Pro Pro Pro Glu Glu Pro Leu
                380
```

<210> 6
<211> 81
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 3815186CD1

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<400> 6
Met Arg Trp Tyr Gln Pro Pro Asn Asp Trp Arg Ile Leu Val Leu
1 5 10 15
Cys Leu Ser Ser Tyr Ala Val Leu Met Cys Leu Leu Ser Ile Trp
20 25 30
Gln Arg Asp Lys Arg Asp Thr Ser Asn Phe Asp Lys Glu Phe Thr
35 40 45
Arg Gln Pro Val Glu Leu Thr Pro Thr Asp Lys Leu Phe Ile Met
50 55 60
Asn Leu Asp Gln Asn Glu Phe Ala Gly Phe Ser Tyr Thr Asn Pro
65 70 75
Glu Phe Val Ile Asn Val
80

<210> 7
<211> 721
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 5504544CD1

<400> 7
Met Leu Phe Gly Leu Val Arg Gln Gly Leu Lys Cys Asp Gly Cys
1 5 10 15
Gly Leu Asn Tyr His Lys Arg Cys Ala Phe Ser Ile Pro Asn Asn
20 25 30
Cys Ser Gly Ala Arg Lys Arg Arg Leu Ser Ser Thr Ser Leu Ala
35 40 45
Ser Gly His Ser Val Arg Leu Gly Thr Ser Glu Ser Leu Pro Cys
50 55 60
Thr Ala Glu Glu Leu Ser Arg Ser Thr Thr Glu Leu Leu Pro Arg
65 70 75
Arg Pro Pro Ser Ser Ser Ser Ser Ser Ser Ala Ser Ser Tyr Thr
80 85 90
Gly Arg Pro Ile Glu Leu Asp Lys Met Leu Leu Ser Lys Val Lys
95 100 105
Val Pro His Thr Phe Leu Ile His Ser Tyr Thr Arg Pro Thr Val
110 115 120
Cys Gln Ala Cys Lys Lys Leu Leu Lys Gly Leu Phe Arg Gln Gly
125 130 135
Leu Gln Cys Lys Asp Cys Lys Phe Asn Cys His Lys Arg Cys Ala
140 145 150
Thr Arg Val Pro Asn Asp Cys Leu Gly Glu Ala Leu Ile Asn Gly
155 160 165
Asp Val Pro Met Glu Glu Ala Thr Asp Phe Ser Glu Ala Asp Lys
170 175 180
Ser Ala Leu Met Asp Glu Ser Glu Asp Ser Gly Val Ile Pro Gly
185 190 195
Ser His Ser Glu Asn Ala Leu His Ala Ser Glu Glu Glu Glu Gly
200 205 210
Glu Gly Gly Lys Ala Gln Ser Ser Leu Gly Tyr Ile Pro Leu Met
215 220 225
Arg Val Val Gln Ser Val Arg His Thr Thr Arg Lys Ser Ser Thr
230 235 240
Thr Leu Arg Glu Gly Trp Val Val His Tyr Ser Asn Lys Asp Thr
245 250 255
Leu Arg Lys Arg His Tyr Trp Arg Leu Asp Cys Lys Cys Ile Thr
260 265 270

```
Leu Phe Gln Asn Asn Thr Thr Asn Arg Tyr Tyr Lys Glu Ile Pro
                275                 280                 285
Leu Ser Glu Ile Leu Thr Val Glu Ser Ala Gln Asn Phe Ser Leu
                290                 295                 300
Val Pro Pro Gly Thr Asn Pro His Cys Phe Glu Ile Val Thr Ala
                305                 310                 315
Asn Ala Thr Tyr Phe Val Gly Glu Met Pro Gly Gly Thr Pro Gly
                320                 325                 330
Gly Pro Ser Gly Gln Gly Ala Glu Ala Ala Arg Gly Trp Glu Thr
                335                 340                 345
Ala Ile Arg Gln Ala Leu Met Pro Val Ile Leu Gln Asp Ala Pro
                350                 355                 360
Ser Ala Pro Gly His Ala Pro His Arg Gln Ala Ser Leu Ser Ile
                365                 370                 375
Ser Val Ser Asn Ser Gln Ile Gln Glu Asn Val Asp Ile Ala Thr
                380                 385                 390
Val Tyr Gln Ile Phe Pro Asp Glu Val Leu Gly Ser Gly Gln Phe
                395                 400                 405
Gly Val Val Tyr Gly Gly Lys His Arg Lys Thr Gly Arg Asp Val
                410                 415                 420
Ala Val Lys Val Ile Asp Lys Leu Arg Phe Pro Thr Lys Gln Glu
                425                 430                 435
Ser Gln Leu Arg Asn Glu Val Ala Ile Leu Gln Ser Leu Arg His
                440                 445                 450
Pro Gly Ile Val Asn Leu Glu Cys Met Phe Glu Thr Pro Glu Lys
                455                 460                 465
Val Phe Val Val Met Glu Lys Leu His Gly Asp Met Leu Glu Met
                470                 475                 480
Ile Leu Ser Ser Glu Lys Gly Arg Leu Pro Glu Arg Leu Thr Lys
                485                 490                 495
Phe Leu Ile Thr Gln Ile Leu Val Ala Leu Arg His Leu His Phe
                500                 505                 510
Lys Asn Ile Val His Cys Asp Leu Lys Pro Glu Asn Val Leu Leu
                515                 520                 525
Ala Ser Ala Asp Pro Phe Pro Gln Val Lys Leu Cys Asp Phe Gly
                530                 535                 540
Phe Ala Arg Ile Ile Gly Glu Lys Ser Phe Arg Arg Ser Val Val
                545                 550                 555
Gly Thr Pro Ala Tyr Leu Ala Pro Glu Val Leu Leu Asn Gln Gly
                560                 565                 570
Tyr Asn Arg Ser Leu Asp Met Trp Ser Val Gly Val Ile Met Tyr
                575                 580                 585
Val Ser Leu Ser Gly Thr Phe Pro Phe Asn Glu Asp Glu Asp Ile
                590                 595                 600
Asn Asp Gln Ile Gln Asn Ala Ala Phe Met Tyr Pro Ala Ser Pro
                605                 610                 615
Trp Ser His Ile Ser Ala Gly Ala Ile Asp Leu Ile Asn Asn Leu
                620                 625                 630
Leu Gln Val Lys Met Arg Lys Arg Tyr Ser Val Asp Lys Ser Leu
                635                 640                 645
Ser His Pro Trp Leu Gln Glu Tyr Gln Thr Trp Leu Asp Leu Arg
                650                 655                 660
Glu Leu Glu Gly Lys Met Gly Glu Arg Tyr Ile Thr His Glu Ser
                665                 670                 675
Asp Asp Ala Arg Trp Glu Gln Phe Ala Ala Glu His Pro Leu Pro
                680                 685                 690
Gly Ser Gly Leu Pro Thr Asp Arg Asp Leu Gly Gly Ala Cys Pro
                695                 700                 705
Pro Gln Asp His Asp Met Gln Gly Leu Ala Glu Arg Ile Ser Val
                710                 715                 720
Leu
```

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<210> 8
<211> 249
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1511326CD1

<400> 8
Met Ala Ser Ser Asp Glu Asp Gly Thr Asn Gly Gly Ala Ser Glu
1 5 10 15
Ala Gly Glu Asp Arg Glu Ala Pro Gly Gln Arg Arg Arg Leu Gly
20 25 30
Phe Leu Ala Thr Ala Trp Leu Thr Phe Tyr Asp Ile Ala Met Thr
35 40 45
Ala Gly Trp Leu Val Leu Ala Ile Ala Met Val Arg Phe Tyr Met
50 55 60
Glu Lys Gly Thr His Arg Gly Leu Tyr Lys Ser Ile Gln Lys Thr
65 70 75
Leu Lys Phe Phe Gln Thr Phe Ala Leu Leu Glu Ile Val His Cys
80 85 90
Leu Ile Gly Ile Val Pro Thr Ser Val Ile Val Thr Gly Val Gln
95 100 105
Val Ser Ser Arg Ile Phe Met Val Trp Leu Ile Thr His Ser Ile
110 115 120
Lys Pro Ile Gln Asn Glu Glu Ser Val Val Leu Phe Leu Val Ala
125 130 135
Trp Thr Val Thr Glu Ile Thr Arg Tyr Ser Phe Tyr Thr Phe Ser
140 145 150
Leu Leu Asp His Leu Pro Tyr Phe Ile Lys Trp Ala Arg Tyr Asn
155 160 165
Phe Phe Ile Ile Leu Tyr Pro Val Gly Val Ala Gly Glu Leu Leu
170 175 180
Thr Ile Tyr Ala Ala Leu Pro His Val Lys Lys Thr Gly Met Phe
185 190 195
Ser Ile Arg Leu Pro Asn Lys Tyr Asn Val Ser Phe Asp Tyr Tyr
200 205 210
Tyr Phe Leu Leu Ile Thr Met Ala Ser Tyr Ile Pro Leu Phe Pro
215 220 225
Gln Leu Tyr Phe His Met Leu Arg Gln Arg Arg Lys Val Leu His
230 235 240
Gly Glu Val Ile Val Glu Lys Asp Asp
245

<210> 9
<211> 146
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1519120CD1

<400> 9
Met Ala Asp Asp Asp Val Leu Phe Glu Asp Val Tyr Glu Leu Cys
1 5 10 15
Glu Val Ile Gly Lys Gly Pro Phe Ser Val Val Arg Arg Cys Ile
20 25 30

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Asn Arg Glu Thr Gly Gln Gln Phe Ala Val Lys Ile Val Asp Val
35 40 45
Ala Lys Phe Thr Ser Ser Pro Gly Leu Ser Thr Glu Gly Lys Arg
50 55 60
Trp Ile Ser Asn Leu Lys Arg Glu Ala Ser Ile Cys His Met Leu
65 70 75
Lys His Pro His Ile Val Glu Leu Leu Glu Thr Tyr Ser Ser Asp
80 85 90
Gly Met Leu Tyr Met Val Phe Glu Phe Met Asp Gly Ala Asp Leu
95 100 105
Cys Phe Glu Ile Val Lys Arg Ala Asp Ala Gly Phe Val Tyr Ser
110 115 120
Glu Ala Val Ala Ser Ile Leu Asp Lys His Ser Trp Lys Gln Leu
125 130 135
Gly Asp His Leu Asn Thr Ala Leu Ser Ser Ala
140 145

<210> 10
<211> 524
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1673761CD1

<400> 10
Met Asn Ile Ala Asn Arg Lys Gln Glu Glu Met Lys Asp Met Ile
1 5 10 15
Val Glu Thr Leu Asn Thr Met Lys Glu Glu Leu Leu Asp Asp Ala
20 25 30
Thr Asn Met Glu Phe Lys Asp Val Ile Val Pro Glu Asn Gly Glu
35 40 45
Pro Val Gly Thr Arg Glu Ile Lys Cys Cys Ile Arg Gln Ile Gln
50 55 60
Glu Leu Ile Ile Ser Arg Leu Asn Gln Ala Val Ala Asn Lys Leu
65 70 75
Ile Ser Ser Val Asp Tyr Leu Arg Glu Ser Phe Val Gly Thr Leu
80 85 90
Glu Arg Cys Leu Gln Ser Leu Glu Lys Ser Gln Asp Val Ser Val
95 100 105
His Ile Thr Ser Asn Tyr Leu Lys Gln Ile Leu Asn Ala Ala Tyr
110 115 120
His Val Glu Val Thr Phe His Ser Gly Ser Ser Val Thr Arg Met
125 130 135
Leu Trp Glu Gln Ile Lys Gln Ile Ile Gln Arg Ile Thr Trp Val
140 145 150
Ser Pro Pro Ala Ile Thr Leu Glu Trp Lys Arg Lys Val Ala Gln
155 160 165
Glu Ala Ile Glu Ser Leu Ser Ala Ser Lys Leu Ala Lys Ser Ile
170 175 180
Cys Ser Gln Phe Arg Thr Arg Leu Asn Ser Ser His Glu Ala Phe
185 190 195
Ala Ala Ser Leu Arg Gln Leu Glu Ala Gly His Ser Gly Arg Leu
200 205 210
Glu Lys Thr Glu Asp Leu Trp Leu Arg Val Arg Lys Asp His Ala
215 220 225
Pro Arg Leu Ala Arg Leu Ser Leu Glu Ser Cys Ser Leu Gln Asp
230 235 240
Val Leu Leu His Arg Lys Pro Lys Leu Gly Gln Glu Leu Gly Arg

245 250 255
Gly Gln Tyr Gly Val Val Tyr Leu Cys Asp Asn Trp Gly Gly His
260 265 270
Phe Pro Cys Ala Leu Lys Ser Val Val Pro Pro Asp Glu Lys His
275 280 285
Trp Asn Asp Leu Ala Leu Glu Phe His Tyr Met Arg Ser Leu Pro
290 295 300
Lys His Glu Arg Leu Val Asp Leu His Gly Ser Val Ile Asp Tyr
305 310 315
Asn Tyr Gly Gly Gly Ser Ser Ile Ala Val Leu Leu Ile Met Glu
320 325 330
Arg Leu His Arg Asp Leu Tyr Thr Gly Leu Lys Ala Gly Leu Thr
335 340 345
Leu Glu Thr Arg Leu Gln Ile Ala Leu Asp Val Val Glu Gly Ile
350 355 360
Arg Phe Leu His Ser Gln Gly Leu Val His Arg Asp Ile Lys Leu
365 370 375
Lys Asn Val Leu Leu Asp Lys Gln Asn Arg Ala Lys Ile Thr Asp
380 385 390
Leu Gly Phe Cys Lys Pro Glu Ala Met Met Ser Gly Ser Ile Val
395 400 405
Gly Thr Pro Ile His Met Ala Pro Glu Leu Phe Thr Gly Lys Tyr
410 415 420
Asp Asn Ser Val Asp Val Tyr Ala Phe Gly Ile Leu Phe Trp Tyr
425 430 435
Ile Cys Ser Gly Ser Val Lys Leu Pro Glu Ala Phe Glu Arg Cys
440 445 450
Ala Ser Lys Asp His Leu Trp Asn Asn Val Arg Arg Gly Ala Arg
455 460 465
Pro Glu Arg Leu Pro Val Phe Asp Glu Glu Cys Trp Gln Leu Met
470 475 480
Glu Ala Cys Trp Asp Gly Asp Pro Leu Lys Arg Pro Leu Leu Gly
485 490 495
Ile Val Gln Pro Met Leu Gln Gly Ile Met Asn Arg Leu Cys Lys
500 505 510
Ser Asn Ser Glu Gln Pro Asn Arg Gly Leu Asp Asp Ser Thr
515 520

<210> 11
<211> 509
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1270442CD1

<400> 11
Met Arg Leu Arg Glu Arg Ser Leu Arg Gln Asp Pro Asp Leu Arg
1 5 10 15
Gln Glu Leu Ala Ser Leu Ala Arg Gly Cys Asp Phe Val Leu Pro
20 25 30
Ser Arg Phe Lys Lys Arg Leu Lys Ala Phe Gln Gln Val Gln Thr
35 40 45
Arg Lys Glu Glu Pro Leu Pro Pro Ala Thr Ser Gln Ser Ile Pro
50 55 60
Thr Phe Tyr Phe Pro Arg Gly Arg Pro Gln Asp Ser Val Asn Val
65 70 75
Asp Ala Val Ile Ser Lys Ile Glu Ser Thr Phe Ala Arg Phe Pro
80 85 90

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```
His Glu Arg Ala Thr Met Asp Asp Met Gly Leu Val Ala Lys Ala
                95                  100                 105
Cys Gly Cys Pro Leu Tyr Trp Lys Gly Pro Leu Phe Tyr Gly Ala
                110                 115                 120
Gly Gly Glu Arg Thr Gly Ser Val Ser Val His Lys Phe Val Ala
                125                 130                 135
Met Trp Arg Lys Ile Leu Gln Asn Cys His Asp Asp Ala Ala Lys
                140                 145                 150
Phe Val His Leu Leu Met Ser Pro Gly Cys Asn Tyr Leu Val Gln
                155                 160                 165
Glu Asp Phe Val Pro Phe Leu Gln Asp Val Val Asn Thr His Pro
                170                 175                 180
Gly Leu Ser Phe Leu Lys Glu Ala Ser Glu Phe His Ser Arg Tyr
                185                 190                 195
Ile Thr Thr Val Ile Gln Arg Ile Phe Tyr Ala Val Asn Arg Ser
                200                 205                 210
Trp Ser Gly Arg Ile Thr Cys Ala Glu Leu Arg Arg Ser Ser Phe
                215                 220                 225
Leu Gln Asn Val Ala Leu Leu Glu Glu Glu Ala Asp Ile Asn Gln
                230                 235                 240
Leu Thr Glu Phe Phe Ser Tyr Glu His Phe Tyr Val Ile Tyr Cys
                245                 250                 255
Lys Phe Trp Glu Leu Asp Thr Asp His Asp Leu Leu Ile Asp Ala
                260                 265                 270
Asp Asp Leu Ala Arg His Asn Asp His Ala Leu Ser Thr Lys Met
                275                 280                 285
Ile Asp Arg Ile Phe Ser Gly Ala Val Thr Arg Gly Arg Lys Val
                290                 295                 300
Gln Lys Glu Gly Lys Ile Ser Tyr Ala Asp Phe Val Trp Phe Leu
                305                 310                 315
Ile Ser Glu Glu Asp Lys Lys Thr Pro Thr Ser Ile Glu Tyr Trp
                320                 325                 330
Phe Arg Cys Met Asp Leu Asp Gly Asp Gly Ala Leu Ser Met Phe
                335                 340                 345
Glu Leu Glu Tyr Phe Tyr Glu Glu Gln Cys Arg Ser Val Asp Ser
                350                 355                 360
Met Ala Ile Glu Ala Leu Pro Phe Gln Asp Cys Leu Cys Gln Met
                365                 370                 375
Leu Asp Leu Val Lys Pro Arg Thr Glu Gly Lys Ile Thr Leu Gln
                380                 385                 390
Asp Leu Lys Arg Cys Lys Leu Ala Asn Val Phe Phe Asp Thr Phe
                395                 400                 405
Phe Asn Ile Glu Lys Tyr Leu Asp His Glu Gln Lys Glu Gln Ile
                410                 415                 420
Ser Leu Leu Arg Asp Gly Asp Ser Gly Gly Pro Glu Leu Ser Asp
                425                 430                 435
Trp Glu Lys Tyr Ala Ala Glu Glu Tyr Asp Ile Leu Val Ala Glu
                440                 445                 450
Glu Thr Ala Gly Glu Pro Trp Glu Asp Gly Phe Glu Ala Glu Leu
                455                 460                 465
Ser Pro Val Glu Gln Lys Leu Ser Ala Leu Arg Ser Pro Leu Ala
                470                 475                 480
Gln Arg Pro Phe Phe Glu Ala Pro Ser Pro Leu Gly Ala Val Asp
                485                 490                 495
Leu Tyr Glu Tyr Ala Cys Gly Asp Glu Asp Leu Glu Pro Leu
                500                 505
```

<210> 12
<211> 142
<212> PRT

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<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1877133CD1

<400> 12
```
Met Ile Ser Thr Ala Arg Val Pro Ala Asp Lys Pro Val Arg Ile
 1               5                  10                  15
Ala Phe Ser Leu Asn Asp Ala Ser Asp Asp Thr Pro Pro Glu Asp
                20                  25                  30
Ser Ile Pro Leu Val Phe Pro Glu Leu Asp Gln Gln Leu Gln Pro
                35                  40                  45
Leu Pro Pro Cys His Asp Ser Glu Glu Ser Met Glu Val Phe Lys
                50                  55                  60
Gln His Cys Gln Ile Ala Glu Glu Tyr His Glu Val Lys Lys Glu
                65                  70                  75
Ile Thr Leu Leu Glu Gln Arg Lys Lys Glu Leu Ile Ala Lys Leu
                80                  85                  90
Asp Gln Ala Glu Lys Glu Lys Val Asp Ala Ala Glu Leu Val Arg
                95                 100                 105
Glu Phe Glu Ala Leu Thr Glu Glu Asn Arg Thr Leu Arg Leu Ala
               110                 115                 120
Gln Ser Gln Cys Val Glu Gln Leu Glu Lys Leu Arg Ile Gln Tyr
               125                 130                 135
Gln Lys Arg Gln Gly Ser Ser
               140
```

<210> 13
<211> 221
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2636759CD1

<400> 13
```
Met Thr Ser Gly Glu Val Lys Thr Ser Leu Lys Asn Ala Tyr Ser
 1               5                  10                  15
Ser Ala Lys Arg Leu Ser Pro Lys Met Glu Glu Glu Gly Glu Glu
                20                  25                  30
Glu Asp Tyr Cys Thr Pro Gly Ala Phe Glu Leu Glu Arg Leu Phe
                35                  40                  45
Trp Lys Gly Ser Pro Gln Tyr Thr His Val Asn Glu Val Trp Pro
                50                  55                  60
Lys Leu Tyr Ile Gly Asp Glu Ala Thr Ala Leu Asp Arg Tyr Arg
                65                  70                  75
Leu Gln Lys Ala Gly Phe Thr His Val Leu Asn Ala Ala His Gly
                80                  85                  90
Arg Trp Asn Val Asp Thr Gly Pro Arg Leu Leu Pro Arg His Gly
                95                 100                 105
His Pro Val Pro Arg Arg Gly Gly Pro Thr Thr Cys Pro Pro Phe
               110                 115                 120
Asp Leu Ser Val Phe Phe Tyr Pro Ala Ala Ala Phe Ile Asp Arg
               125                 130                 135
Ala Leu Ser Asp Asp His Ser Lys Ile Leu Val His Cys Val Met
               140                 145                 150
Gly Arg Ser Arg Ser Ala Thr Leu Val Leu Ala Tyr Leu Met Ile
               155                 160                 165
```

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```
His Lys Asp Met Thr Leu Val Asp Ala Ile Gln Gln Val Ala Lys
                170                 175                 180
Asn Arg Cys Val Leu Pro Asn Arg Gly Phe Leu Lys Gln Leu Arg
                185                 190                 195
Glu Leu Asp Lys Gln Leu Val Gln Gln Arg Arg Arg Ser Gln Arg
                200                 205                 210
Gln Asp Gly Glu Glu Glu Asp Asp Arg Glu Leu
                215                 220


<210> 14
<211> 462
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2716815CD1

<400> 14
Met Ser Ile Ala Gly Val Ala Ala Gln Glu Ile Arg Val Pro Leu
  1               5                  10                  15
Lys Thr Gly Phe Leu His Asn Gly Arg Ala Met Gly Asn Met Arg
                 20                  25                  30
Lys Thr Tyr Trp Ser Ser Arg Ser Glu Phe Lys Asn Asn Phe Leu
                 35                  40                  45
Asn Ile Asp Pro Ile Thr Met Ala Tyr Ser Leu Asn Ser Ser Ala
                 50                  55                  60
Gln Glu Arg Leu Ile Pro Leu Gly His Ala Ser Lys Ser Ala Pro
                 65                  70                  75
Met Asn Gly His Cys Phe Ala Glu Asn Gly Pro Ser Gln Lys Ser
                 80                  85                  90
Ser Leu Pro Pro Leu Leu Ile Pro Pro Ser Glu Asn Leu Gly Pro
                 95                 100                 105
His Glu Glu Asp Gln Val Val Cys Gly Phe Lys Lys Leu Thr Val
                110                 115                 120
Asn Gly Val Cys Ala Ser Thr Pro Pro Leu Thr Pro Ile Lys Asn
                125                 130                 135
Ser Pro Ser Leu Phe Pro Cys Ala Pro Leu Cys Glu Arg Gly Ser
                140                 145                 150
Arg Pro Leu Pro Pro Leu Pro Ile Ser Glu Ala Leu Ser Leu Asp
                155                 160                 165
Asp Thr Asp Cys Glu Val Glu Phe Leu Thr Ser Ser Asp Thr Asp
                170                 175                 180
Phe Leu Leu Glu Asp Ser Thr Leu Ser Asp Phe Lys Tyr Asp Val
                185                 190                 195
Pro Gly Arg Arg Ser Phe Arg Gly Cys Gly Gln Ile Asn Tyr Ala
                200                 205                 210
Tyr Phe Asp Thr Pro Ala Val Ser Ala Ala Asp Leu Ser Tyr Val
                215                 220                 225
Ser Asp Gln Asn Gly Gly Val Pro Asp Pro Asn Pro Pro Pro Pro
                230                 235                 240
Gln Thr His Arg Arg Leu Arg Arg Ser His Ser Gly Pro Ala Gly
                245                 250                 255
Ser Phe Asn Lys Pro Ala Ile Arg Ile Ser Asn Cys Cys Ile His
                260                 265                 270
Arg Ala Ser Pro Asn Ser Asp Glu Asp Lys Pro Glu Val Pro Pro
                275                 280                 285
Arg Val Pro Ile Pro Pro Arg Pro Val Lys Pro Asp Tyr Arg Arg
                290                 295                 300
Trp Ser Ala Glu Val Thr Ser Ser Thr Tyr Ser Asp Glu Asp Arg
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Pro Pro Lys Val Pro Pro Arg Glu Pro Leu Ser Pro Ser Asn Ser
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                    335                         340                         345
Met Pro Pro Thr Gln Ser Phe Ala Pro Asp Pro Lys Tyr Val Ser
                    350                         355                         360
Ser Lys Ala Leu Gln Arg Gln Asn Ser Glu Gly Ser Ala Ser Lys
                    365                         370                         375
Val Pro Cys Ile Leu Pro Ile Ile Glu Asn Gly Lys Lys Val Ser
                    380                         385                         390
Ser Thr His Tyr Tyr Leu Leu Pro Glu Arg Pro Pro Tyr Leu Asp
                    395                         400                         405
Lys Tyr Glu Lys Phe Phe Arg Glu Ala Glu Glu Thr Asn Gly Gly
                    410                         415                         420
Ala Gln Ile Gln Pro Leu Pro Ala Asp Cys Gly Ile Ser Ser Ala
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<213> Homo sapiens

<220>
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<222> 959, 1029, 1159
<223> a or g or c or t, unknown, or other

<220>
<221> misc_feature
<223> Incyte ID No: 563663CB1

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agaagcagac ctgggaatca cctaacatgt cgaggaggag atttgattgc cgaagtattt  240
caggcctact aactacaact cctcaaattc caataaaaat ggaaaacttt aataatttct  300
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caaaatctac tggccaagaa tatgctgcaa aatttctaaa aaagagaaga agaggacagg  420
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gtgttattaa tcttcatgag gtctatgaaa atacaagtga aatcattttg atattggaat  540
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<210> 17
<211> 1706
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 1425842CB1

<400> 17

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<212> DNA
<213> Homo sapiens

<220>
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<212> DNA
<213> Homo sapiens

<220>
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<223> Incyte ID No: 2415617CB1

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<212> DNA
<213> Homo sapiens

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<223> Incyte ID No: 3815186CB1

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<212> DNA
<213> Homo sapiens

<220>
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Filed on	9 July 1999 (09.07.1999)
US	60/125,593 (CIP)
Filed on	18 March 1999 (18.03.1999)

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/55332 A3

(54) Title: HUMAN REGULATORS OF INTRACELLULAR PHOSPHORYLATION

(57) Abstract: The invention provides human regulators of intracellular phosphorylation (HRIP) and polynucleotides which identify and encode HRIP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HRIP.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/07277

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N15/55 C12N9/12 C12N9/16 C07K16/18
C07K16/40 C12Q1/68 A61K38/45 A61K38/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 39446 A (HUMAN GENOME SCIENCES, INC.) 11 September 1998 (1998-09-11) abstract page 1, line 1 -page 2, line 8 page 62, line 6 - line 29 page 87, line 10 -page 93, line 25 page 98, line 1 -page 101, line 6 page 365 -page 366 page 381; claims 1,3 -& GCG_GENESEQ_D database, accession number V59579 6 January 1999 "Human secreted protein gene 69 clone HETGJ09" XP002149750 the whole document --- -/--	10-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2000	11 JAN 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

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Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 00/07277

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	TANOUE, T. ET AL.: "Molecular Cloning and Characterziation of a Novel Dual Specificity Phosphatase, MKP-5" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 28, 9 July 1999 (1999-07-09), pages 19949-19956, XP002148678 the whole document ---	1-3,5,6, 8-14
A	GROOM, L.A. ET AL.: "Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specific phosphatase" EMBO JOURNAL, vol. 15, no. 14, 15 July 1996 (1996-07-15), pages 3621-3632, XP000925967 the whole document ---	1-17,20, 23
A	WO 99 01541 A (TULARIK INC.) 14 January 1999 (1999-01-14) abstract page 4, line 10 -page 16, line 13 page 17 -page 18; claims 1-11 ---	1-17,20, 23
A	WO 99 00507 A (INCYTE PHARMACEUTICALS, INC.) 7 January 1999 (1999-01-07) abstract page 2, line 13 -page 3, line 31 page 38, line 10 -page 46, line 10 page 55 -page 57; claims 1-21 -----	1-17,20, 23

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/07277

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 16 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 18, 19, 21 and 22
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-17, 20, 23 partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-17, 20, 23 partially

Invention 1

An isolated polypetide comprising an amino acid sequence selected from the group consisting of: a) an amino acid sequence having the SEQ ID NO: 1, b) a naturally occurring amino acid sequence having at least 90% sequence identity to SEQ ID NO: 1, c) a biologically active fragment of SEQ ID NO: 1, d) an immunogenic fragment of SEQ ID NO: 1; an isolated polynucleotide encoding said polypeptide; a recombinant polynucleotide comprising said polynucleotide; a cell transformed with said recombinant polynucleotide; a transgenic organism comprising said recombinant polynucleotide; a method for producing said polypeptide; an isolated antibody which specifically binds to said polypeptide; an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of: a) a polynucleotide sequence having the SEQ ID NO: 15, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 15, c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); an RNA equivalent of a)-d); a method for detecting a target polynucleotide in a sample having the sequence of said polynucleotide by hybridizing with a probe; a pharmaceutical composition comprising an effective amount of said polypeptide; a method for treating a disease or condition associated with decreased expression of functional HRIP, comprising administering to a patient said pharmaceutical composition; a method for screening a compound for effectiveness as an agonist or antagonist of said polypeptide; a method for screening a compound for effectiveness in altering expression of a polynucleotide sequence having the SEQ ID NO: 15;

2. Claims: 1-17, 20, 23 partially

Invention 2

Idem as subject 1 but limited to SEQ ID NOS: 2 and 16;

3.-14. Claims: 1-17, 20, 23 partially

Inventions 3-14

Idem as subject 1 but limited to SEQ ID NOS: 3-14 and 17-28.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 18, 19, 21 and 22

Claims 18, 19, 21 and 22 refer to an agonist and an antagonist of a polypeptide of claim 1 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).
No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/07277

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9839446 A	11-09-1998	AU 6545398 A	22-09-1998
		EP 0972029 A	19-01-2000
		EP 0972030 A	19-01-2000
		WO 9839448 A	11-09-1998
WO 9901541 A	14-01-1999	AU 726294 B	02-11-2000
		AU 8283698 A	25-01-1999
		EP 1005539 A	07-06-2000
WO 9900507 A	07-01-1999	US 5955338 A	21-09-1999
		AU 8269998 A	19-01-1999
		EP 0996733 A	03-05-2000